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FINAL No.1 *REPORT ON*

CONTRACT NO DA 92-557-FEC-35775

INCLUSIVE DATES 20 January 1962 *TO* 19 January 1963

SUBJECT OF INVESTIGATION

Genetic, serologic,
and
biochemical studies
on
viral infection and lysogenization

RESPONSIBLE INVESTIGATOR

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Office of the Chief of Research and Development
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ABSTRACT

Evidences are presented that lysogenization is affected by viral genetic properties and by the presence of the genome of a second unrelated phage. Rapid establishment of lysogeny of phage ε^{34} is attributed to viral genetic properties. Lysogenization with ε^{34} is interfered by prophage ε^{15} but not by prophage ε^{15}. Prophage establishment of phage g_{341} is completely suppressed in cells A(ε^{34}) but not in cells A. At low m.o.i. phage ε^{15}[A] is interfered with prophage establishment in cells A(ε^{34}) while not at high m.o.i.. Even at low m.o.i. ε^{15}[A(ε^{34})] establishes lysogeny in A(ε^{34}) without interference. There is no interference in infection of strains A and A(g_{341}) with ε^{15}[A] and/or ε^{15}[A(g_{341})] and in infection of strains A and A(ε^{y}) with g_{341}[A]. Most probable explanation to these findings would be that lysogenization may be affected by host-controlled variation (HCV).

Phage ε^{15} goes under HCV. Cells I-1 which do not allow multiplication of phage ε^{15}[A] by single infection may allow phage growth when multiply infected ("Multiplicity activation" = MA). Phage ε^{15}ts[A] is capable of contributing to MA in infection of I-1 with ε^{15}[A] while ε^{15}ts[I-1] or UV-inactivated ε^{15}[A] takes little or no MA effect for ε^{15}[A], indicating that the helper effect is influenced by minor difference(s) in DNA structure. Factor(s) responsible for HCV proved to be thermolabile, highly specific, and capable of interacting with intact phage DNA.

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Analysis of lipopolysaccharides with specificity of O antigen 3 prepared from Salmonella senftenberg 37Aa showed that galactose, mannose, and rhamnose are main constituents.

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CONTENTS

1. Purpose of researches

In the process of viral infection and lysogenization, there are many phenomena, of which mechanisms remain to be elucidated. These may be analysed genetically, serologically, and biochemically.

We have been working on antigenic conversion, viral infection, and lysogenic conversion since 1949, and main important findings are as summarized in application paper. Our system of research consists of Salmonella strains of group E and temperate converting phages carrying genes responsible for the synthesis of somatic antigens 15 and/or 34 of host bacterial cells.

Basing upon the findings obtained, and by making use of the lysogenic conversion system mentioned above, researches are being carried out in order to elucidate genetic and chemical factors involved in the process of viral infection and in the process of lysogenization. And in practice the following research works have been designed to be carried out.

a. Analysis of factors affecting on the process of lysogenization

b. Studies on enzymic activities in cells destined to lysogeny after phage infection

c. Studies on the mechanism of host-controlled variation (HCV)

And during past one year, the following results were obtained.

2. Analysis of factors affecting on the process of lysogenization

The process of lysogenization is different between phages ε^{15} and ε^{34}; it takes approximately 3 hours for ε^{15} to establish lysogeny (Uetake et al., 1958), while ε^{34} usually becomes a prophage very rapidly after infection (Hagiwara, 1959a, 1959b; Uetake and Hagiwara, 1959). Some phages resemble to phage ε^{15} and others to phage ε^{34} in establishing lysogeny (see Bertani, 1958).

However, it seems premature to conclude that the difference is due to genetic difference(s) between the two phages, since the host cell of ε^{15}, A, is different from that of ε^{34}, A(ε^{15}), in that the latter carries prophage ε^{15}. Hence, the question remains to be determined whether or not the presence of phage genome(s) affects the process of lysogenization of a second unrelated phage. From such a viewpoint, effects of the presence of phage genome(s) upon the process of lysogenization of a second unrelated phage were studied experimentally. And this has eventually elucidated a close relationship between lysogenization and host-controlled variation as described below.

a. Materials and Methods

(1) Bacterial strains: Salmonella anatum 293 (=A) and its lysogenic derivatives, A(ε^{15}), A(ε^{34}), A(g_{341}), and A(ε^{y}) have been described elsewhere (Uchida et al., 1956; Uetake et al., 1958; Uetake and Uchida, 1959; Uetake and Hagiwara, 1960a, 1960b, 1961).

(2) Phage strains: Temperate phages ε^{15}, ε^{34}, and g_{341} have been described (Uetake et al., 1955; Uetake, 1956; Nakagawa, 1957a, 1957b, 1959; Uchida et al., 1956). Phage ε^{15} which is propagated on strain A or strain A(g_{341}) is described as ε^{15}[A] and or ε^{15}[A(g_{341})], respectively.

(3) Antiphage sera have also been described (Uetake et al., 1958). The neutralization velocity constants of anti-ε^{15}, anti-g_{341}, and anti-ε^{34} sera are 120 min^{-1}, 180 min^{-1}, and 130 min^{-1}, respectively.

(4) Follow-up of the process of lysogenization: Growing cells were infected with phage, and thereafter surviving cells were plated out on agar plates at appropriate time intervals. Resulting colonies were tested for phage production by replicating onto plates seeded with indicator cells (Lederberg and Lederberg, 1952; Uetake et al., 1958; Hagiwara, 1959a). This type of experiments were carried out in various combinations of phage and bacterial strains and at various multiplicities of infection.

b. Lysogenization with phage ε^{34} of cells without carrying prophage ε^{15}. To test the effects of prophage ε^{15} on lysogenization process with ε^{34}, cells A without carrying prophage ε^{15} were infected with ε^{34}. To carry out these experiments special design of experiment was made because cells A do not adsorb phage ε^{34} at all.

When cells A are infected with ε^{15} at a multiplicity of infection (m.o.i.) of about 5, cells which survive infection segregate ε^{15}-carrier and non-carrier cells in their progeny. At about 3 hours after infection, about 80% of cells in the culture possess receptors for ε^{34} without carrying ε^{15} (Uetake et al., 1958). Therefore, by infecting the non-carrier progenies with receptors for ε^{34} by ε^{34}, cells A might probably be lysogenized with ε^{34} without carrying ε^{15} (Uetake and Hagiwara, 1960a, 1960b, 1961). In practice, this was carried out as follows.

Growing cells of A were infected with ε^{15} at m.o.i. of 7.9. After 10 minute adsorption, the mixture received anti-phage serum to remove unadsorbed phages. After another 10 minutes, a dilution was made into broth containing anti-ε^{15} serum, and kept at 37°C without aeration. After 3 hours, cells were chilled, collected by centrifugation, resuspended in broth at a density of about 10^{8} cells per ml, and infected

with ε^{34} at m.o.i. of 1.8. After 10 minute adsorption and another 10 minute treatment with anti-ε^{34} serum, a dilution was made into broth containing anti-ε^{34} serum, and kept at 37°C for 6 hours, during which time cells were plated out on agar plates for viable count and resulting colonies were replicated on both plates seeded with cells A and those with cells A(ε^{15}) to test for production of phage ε^{15} and ε^{34}, respectively.

Approximately 50% of cells survived infection with ε^{34}. Among survivors about 80% were ε^{34}-producers at first, 70% after 60 minutes, 60% after 90 minutes and thereafter, indicating that ε^{34} establishes lysogeny within 60-90 minutes. This result is consistent with those in experiments in which strain A(ε^{15}) was infected with ε^{34} (Hagiwara, 1959a, 1959b, 1959c; Uetake and Hagiwara, 1959). Therefore, it can be concluded that phage ε^{34} establishes lysogeny soon after infection, irrespective of the presence of prophage ε^{15}. In other words, it indicates that rapid establishment of lysogeny in ε^{34}-infected cells is mainly attributed to genetic properties of phage ε^{34}.

c. Interference with establishment of lysogeny of phage ε^{15} in cells carrying prophage ε^{34}. To test effects of prophage ε^{34} on lysogenization with phage ε^{15}, cells A(ε^{34}), which were obtained as described above (Uetake and Hagiwara, 1960a, 1960b, 1961), were infected with ε^{15}[A] at m.o.i. of 6-9 and the process of lysogenization was followed up as described. The results of one of these experiments is shown in Fig. 1.

In strain A as control, lysogenization was established in about 3 hours after infection, consistent with the experiments reported by Uetake et al. (1958), but in cells A(ε^{34}), lysogenization with ε^{15}[A] was so difficult that surviving cells continued to segregate ε^{15}-carrier and non-carrier cells in their progeny even until 6 hours after infection. This seems to indicate that the existence of prophage ε^{34} in cells A interfers with phage ε^{15} to establish lysogeny, and for explanation following possibilities may be considered: (1) Some metabolic changes in cells due to the presence of ε^{34} may be responsible; (2) efficiency of plating (EOP) of ε^{15}[A] on A(ε^{34}) is about 1/3 of that on A, suggesting host-controlled variation (HCV) (Uetake et al. in press), and the HCV may possibly be correlated with the difficulty of lysogenization; (3) steric hindrance due to the attachment of ε^{34} genome to bacterial chromosome may be responsible.

d. Test for the hypothesis (1). To test the hypothesis (1), cells of A were infected with ε^{15} and 30 minutes later superinfected with ε^{34}. In this case, phage ε^{15} established itself as a prophage in 180 minutes as seen in the case without ε^{34}-superinfection (Uetake et al., 1958), while phage ε^{34} had difficulty in establishing lysogeny and non-ε^{34} carrying progeny cells were segregated out from ε^{34}-infected cells for longer than 3 hours.

It can be said, from the aboves that lysogenization with ε^{15} is remarkably affected by prophage ε^{34} but not by ε^{34} when it is in prelysogenic state, and, in addition, that phage ε^{34} becomes a prophage very easily and rapidly in cells carrying prophage ε^{15} and/or no ε^{15}, while lysogenization with ε^{34} is interfered in freshly ε^{15}-infected cells in which ε^{15} is still in prelysogenic state. And it seems unlikely that the metabolic changes due to genetic functions of phage ε^{34} greatly affect the lysogenization process with ε^{15}.

e. Tests for the hypotheses (2) and (3). To test the hypotheses (2) and (3), cells A(ε^{34}) were infected with ε^{15}, which were propagated on cells of A(ε^{34}), at m.o.i. of about 5-10, instead of ε^{15} propagated on cells A. As shown in Table 1, ε^{15} established lysogeny in about 3 hours as phages ε^{15}[A] do in infection of cells A, indicating that lysogenization is affected by phage propagating strains, i.e. probably host-controlled variation. This also indicates, on the other hand, that the interference with prophage establishment of ε^{15}[A] in A(ε^{34}) is not attributed to the steric hindrance due to the presence of prophage ε^{34}, excluding the possibility of the hypothesis (3).

f. Effect of multiple infection upon establishment of lysogeny of phage ε^{15}[A] in cells of A(ε^{34}). As described in Section 3, experiments of HCV which were carried out in parallel with these experiments showed that multiple infection with restricted phages may lead to phage multiplication even in cells which do not allow phage growth when singly infected.

Basing upon these findings, effect of multiple infection on establishment of lysogeny of ε^{15}[A] in cells of A(ε^{34}) was tested. As shown in Table 1 and Fig. 2, when cells of A(ε^{34}) were infected with ε^{15}[A] at high m.o.i. (20.0; 24.2), establishment of prophage state of ε^{15} was completed in about 3 hours after infection without interference. This also favors the hypothesis (2).

g. Lysogenization of strains A and A(g_{341}) with ε^{15}[A] and/or ε^{15}[A(g_{341})]. There is no HCV when phage ε^{15} is propagated on A and on A(g_{341}). If HCV were responsible for the interference with prophage establishment, no interference would be expected in this system. And this proved to be true in the following experiments.

Each of bacterial strains was infected with either ε^{15}[A] or ε^{15}[A(g_{341})] at m.o.i. of about 5, and the process of lysogenization with ε^{15} was followed up with results shown in Figs. 1 and 3.

Phage ε^{15}[A] lysogenizes strain A in 3 hours and strain A(g_{341}) in 4 hours, whereas ε^{15}[A(g_{341})] lysogenizes strain A in 4 hours and strain A(g_{341}) in 3 hours. However, the difference between 3 and 4 hours seems too small to be regarded as significant.

h. Lysogenization of strains A and A(ε^{y}) with g_{341}[A]. There is

no HCV either in phage g_{341} when plated on A and on A(ε^y). and similar result to the above g was obtained in this system, too.

In either bacterial strain, phage infection was carried out at m.o.i. of 0.8-4.8. As shown in Figs. 4 and 5, in either strain g_{341}[A] established lysogeny in 60-90 minutes after infection. There was no difference between the two strains indicating that prophage ε^y has no effect on lysogenization with g_{341}.

i. Infection of A(ε^{34}) with phage g_{341}. The strain A(ε^{34}) is resistant to infection with phage C_{341}, a virulent mutant of g_{341}, although it adsorbs C_{341} to a considerable extent (Uetake and Hagiwara, 1960a, 1960b, 1961). The same was true in infection with g_{341}. Adsorption of g_{341} to A(ε^{34}) is irreversible but there is neither killing of cells to a demonstrable extent nor lysogenization.

j. Discussion. From the above experiments it should be pointed out that the presence of phage genome(s) may affect on the process of lysogenization of a second unrelated phage. An extreme case is a complete suppression of multiplication and lysogenization of phage g_{341} in A(ε^{34}), but more interesting is a relationship between ε^{15} and ε^{34}. The experiments described in b and c indicate that the rapid establishment of the prophage state of phage ε^{34} is attributed to genetic properties of the phage, being not correlated to the presence of the prophage ε^{15} in cells A. On the other hand, these experiments also indicate that the effect of ε^{15} genome on ε^{34} varies with the state of ε^{15} genome. When it is in prelysogenic state, ε^{34} is excluded.

Phage ε^{15} is interfered with establishment of lysogeny in cells carrying prophage ε^{34} as shown in experiments c and the mechanism is regarded to be due to HCV as shown in d, e, f, g and h. Efficiencies of plating of phages ε^{15} and g_{341} propagated on various strains, are summarized in Table 2. Comparing Table 2 with the above experimental results, one can notice a remarkable correlation between HCV and interference with lysogenization. The interference is observed only in combination of phage and bacterial strains in which HCV is also observed. And even in such a combination, infection of cells with restricted phages at high m.o.i. may lead to prophage establishment without interference (f). This is consistent with a phenomenon "Multiplicity activation (MA)" observed in experiments on the mechanism of HCV. (see Section 3), favoring the explanation proposed.

On the one hand, phages ε^{15}[A(ε^{34})] show higher EOP on cells A than on cells A(ε^{34}), and on the other hand, lysogenization of cells A with ε^{15}[A(ε^{34})] is completed in 3 hours even at low m.o.i. (e) as seen in infection of cells A with ε^{15}[A]. This also favors our hypothesis.

However, there still remains a following possibility to be excluded. The higher the m.o.i., the more frequent would be the collisions be-

tween phage DNA and bacterial chromosome, leading to more rapid lysogenization. But this should not be the case, because in infection of cells A with ε^{15}[A] lysogenization is completed in 3 hours even at as low m.o.i. as 1 (Table 1).

The continuation for a long time of segregation of sensitive progeny cells from ε^{15}[A]-infected cells of A(ε^{34}), suggests that injected phage genome persists in the cytoplasm without replication. And both in infection of A with ε^{15}[A] and in infection of A(ε^{34}) with ε^{15}[A(ε^{34})], phage genome seems to persist in preprophage state for some time before establishing prophage state. Nevertheless the preprophage state persists for a longer time in the former case than in the latter. What is the explanation?

If the structure of DNA of ε^{15}[A] is identical with that of ε^{15}[A(ε^{34})], the aboves should not be expected. These may rather suggest that DNA of ε^{15}[A] and that of ε^{15}[A(ε^{34})] have different structures, each of which is fit for attaching to bacterial chromosome of A or A(ε^{34}), respectively. If so, such structures must be produced after multiplication of ε^{15} on A and or A(ε^{34}), respectively. Then, if DNA of ε^{15}[A] is replicated following infection of A(ε^{34}), de novo replicated DNA must have a structure fit for attaching to chromosome of A(ε^{34}). High multiplicity infection (f) may result in replication of injected phage DNA and eventually lead to unimpaired establishment of lysogeny. The fact that ε^{15}[A(ε^{34})] has a higher EOP on A than A(ε^{34}) may indicate that ε^{15}[A(ε^{34})] replicates and multiplies more easily in A than in A(ε^{34}), and in this case lysogenization of A with ε^{15}[A] proceeds without retardation.

In short, by assuming that the structure of phage DNA is modified to some extent by the host cell on which it is propagated and this modification reflects on the ability to replicate in bacterial strains to be infected, and that when not easily replicated, infecting phage DNA maintains its structure as it is and has difficulty to establish a prophage state, while when easily replicated, de novo replicated DNA takes a structure fit to a new host cell, having no difficulty in establishing lysogeny, all of the above data can be explained clearly and in addition these assumptions are compatible with our findings in experiments on the mechanism of HCV (see Section 3) and recent reports on phage λ (Arber and Dussoix, 1962; Dussoix and Arber, 1962). And the replication of phage DNA before establishment of prophage state has been suggested in phages λ (Stent and Fuerst, 1956), P22 (Luria et al., 1958), and P2 (Bertani, 1962). In phage P2 lysogenization is not affected by m.o.i. and may be multiple even after single infection, and this may be easily explained by assuming that P2 DNA replicates very easily and immediately after injection (Bertani, 1962).

k. Conclusions. Phage ε^{15}[A] establishes lysogeny in about 3 hours in cells A, whereas it takes longer than 6 hours in cells A(ε^{34}) when infected at low m.o.i.. On the other hand, lysogenization with ε^{15}

can be completed in 3 hours even in cells A(ε^{34}) when infected with ε^{15}[A] at high m.o.i. and/or with ε^{15}[A(ε^{34})] at low m.o.i..

In contrast, there is no interference with lysogenization in infection of strains A and A(g_{341}) with ε^{15}[A] and/or ε^{15}[A(g_{341})] and in infection of strains A and A(ε^{y}) with g_{341}[A].

Most probable explanation to these findings would be that the process of lysogenization may be affected by host-controlled variation of the infecting phage of which the mechanism was discussed in detail.

In addition, it has also been shown that the rapid establishment of lysogeny with ε^{34} is attributed to genetic properties of phage ε^{34}.

3. Studies on the mechanism of host-controlled variation (HCV)

Phages ε^{15} and ε^{34} go under HCV. HCV is a well-known phenomenon but its exact mechanism is still obscure (Felix and Anderson, 1951; Anderson and Felix, 1952, 1953; Luria and Human, 1952; Ralston and Krueger, 1952; Bertani and Weigle, 1953; Luria, 1953; Garen and Zinder, 1955; Maio and Zahler, 1958; Arber and Lataste-Dorolle, 1961; Christensen, 1961, 1962; Drexler and Christensen, 1961; Arber and Dussoix, 1962; Dussoix and Arber, 1962). Basing upon suggestions which we got recently, the analysis of the mechanism has been carried out.

a. Materials and Methods

(1) Bacterial strains: Salmonella anatum 293 (=A), and its derivative A(ε^{15}) are described in Section 2. Salmonella butantan (=I-1) is an international standard strain of group E_1 Salmonella.

(2) Phage strains: Temperate phage ε^{15}, its virulent mutant ε^{15}vir (Uetake et al., 1958) and its temperature sensitive mutant ε^{15}ts (see Section 4) were employed.

(3) Anti-ε^{15} serum is described in Section 2.

(4) Serological tests: Antibacterial antisera, anti-10 and anti-15 sera were prepared by routine procedures, and slide and tube agglutination tests were done as usual (Kauffmann, 1954).

(5) Tests for lysogenicity was done by replica plating (Lederberg and Lederberg, 1952; Uetake et al., 1958; Hagiwara, 1959a).

(6) Ultraviolet light irradiation: Growing cells from aerated broth culture were resuspended in phosphate buffer (pH 7.0; M/150). Samples were irradiated in Petri dishes at 80 cm distance from a germicidal lamp (Matsuda GR 1510, 15W).

b. Dependence on propagating strains of EOP. Phage ε^{15} propagated on cells of A shows EOP of about 10^{-2} on cells of I-1, while ε^{15} propagated on cells I-1 shows EOP of 10^{-4} on cells A (Table 3). Phages contained in a single plaque on one strain shows similarly lower EOP on the other strain. Single cycle growth of the phage on one strain results in production of phages with lower EOP on the other strain (Table 4).

This reciprocal shifting of EOP proved experimentally to be due to HCV and not to host range mutation by the following experiments.

c. Phage adsorption and injection of phage genetic materials into the host cell. Regardless of the difference in EOP, phages can be adsorbed well even onto cells with lower EOP as shown in Table 3.

Since it has been shown that when cells A are infected with ε^{15}, de novo synthesis of somatic antigen 15 on phage-infected cells can be demonstrated several minutes after infection (Uetake et al., 1958), injection of phage genetic material into cells can be tested by de novo synthesis of phage-controlled antigens.

Growing cells of A and of I-1 were infected with either ε^{15}[A] or ε^{15}[I-1] at m.o.i. of 5-10, and 25 and 50 minutes thereafter, aliquots were removed and heated at 100°C for 4 minutes. After centrifugation cells were resuspended in 0.2% saline and tested for antigen 15 by tube agglutination. As shown in Table 5, in any of combinations of bacterial and phage strains de novo synthesis of somatic antigen 15 was demonstrated, indicating the injection of phage DNA into bacterial cells.

d. Cell killing and lysogenization. Cells A and I-1 were infected with ε^{15}[A] at m.o.i. of about 9. After 10 minute adsorption and another 10 minute treatment with anti-ε^{15} serum, cells were plated out onto agar plates for viable count, and resulting colonies were tested for lysogenicity by replica plating.

As shown in Table 6, more cells were killed in I-1 (92.6%) than in A (66.4%), whereas lysogenics among survivors were 99.8% in A and 30% in I-1.

Similar experiments were made in infection of cells A and I-1 with ε^{15}[I-1]. Surviving cells were 100% in A and 15% in I-1. Lysogenics among survivors were 60.5% in I-1 but not to a demonstrable extent in A (Table 6).

These indicate that in infection with restricted phages infection leading to abortion and curing is very frequent.

e. Serological properties of the restricted phage. Phage ε^{15}[I-1] is neutralized to the same extent as ε^{15}[A] by antiserum prepared against ε^{15}[A].

f. Mixed infection of cells I-1 with ε^{15}[A] and ε^{15}vir[A]. When cells I-1 were mixedly infected with ε^{15}[A] and ε^{15}vir[A], number of mixed yielders was far more than that calculated under the assumption that each of ε^{15}[A] and ε^{15}vir[A] contains a host range mutant capable of attacking cells I-1 at a proportion of about 10^{-2} and 2×10^{-2}, respectively, favoring our hypothesis that the difference in EOP should not be attributed to h mutant but to HCV (Table 7).

g. Physiological states of cells. UV irradiation of cells did not affect EOP at all. However, when cells of I-1, which were grown under aeration for about 5 hours after entering stationary phase of growth, were infected with restricted phage ε^{15}[A] at m.o.i. of 0.02, EOP increased to 10^{-1}, showing the dependence of EOP on the physiological states of bacterial cells (see also MA in synthetic medium).

h. Effect on EOP of multiple infection. During the course of the above experiments, the data were found which suggest that multiple infection may lead to phage multiplication even in cells which do not allow phage growth when singly infected. This was studied in detail.

Cells of I-1 were infected with ε^{15}[A] at various m.o.i., ranging from 0.03 to 16.5, and numbers of infective centers and killed cells were examined.

In experiments in nutrient broth, as shown in Fig. 6, proportion of plaque formers to phage-infected cells increases with increasing m.o.i., reaching 1.0 at m.o.i. of about 10. The data in Fig. 6 also indicate that about 5 particles per cell are required for phage multiplication. Essentially the same results were also obtained in experiments carried out in synthetic medium, and the only difference was in that fewer particles were sufficient to induce phage multiplication (Fig. 7).

i. Cell killing and lysogenization after multiple infection. In complete paralell to the above "Multiplicity activation", it was also shown that number of cells I-1 killed by ε^{15}[A] increases with increasing m.o.i., reaching to more than 99% at m.o.i. of 10 or more (Fig. 8).

The proportion of lysogenics among surviving cells also increases with increasing m.o.i. as shown in Fig. 9. But the proportion of lysogenic cells to input cells were not variable, ranging from 1.6% to 2.4%.

These findings indicate that it is the number of killed or lysing cells that increases with increasing m.o.i.. In other words, multiple infection may lead to phage multiplication and lysis in cells which do not allow phage growth when singly infected.

j. Effect of UV-inactivated phage. Multiplicity reactivation is a well-known phenomenon in UV-inactivated phages (Luria, 1947; Luria and Dulbecco, 1949; Harm, 1956; Tessman and Ozaki, 1957; Epstein, 1958;

Baricelli, 1960). The question was to be determined if a similar mechanism is involved in MA. Phages which were inactivated to 0.06% survival by UV irradiation were tested for their ability to contribute to MA, but positive results were obtained in neither ε^{15}[A] nor ε^{15}[I-1]. So it seemed likely that MA is different from multiplicity reactivation in its mechanism.

On the other hand, this experiment led us to the suspision that the spacial configuration of UV-inactivated phage DNA may be different from that of the native one and it is responsible for the inability to contribute to MA. To test this, ε^{15}ts was employed. Phage ε^{15}ts is able to multiply at 25°C but not at 37°C.

k. Contribution of ε^{15}ts[A] to MA. When growing cells of I-1 were mixedly infected at 37°C with ε^{15}[A] and ε^{15}ts[A] at m.o.i. of about 0.015 and 9 respectively, 99% of ε^{15}[A]-infected cells gave rise to plaques, while without adding ε^{15}ts[A] only 1% of ε^{15}[A]-infected cells were plaque formers. And in addition, when phages ε^{15}ts[I-1] were employed in place of ε^{15}[A] in the above experiment, about 35% of ε^{15}[A]-infected cells were plaque formers, probably being due to genetic recombination.

These results suggest that to give rise to MA, phage DNA should be intact and propagating strain-specific.

l. Infection of A(ε^{34}) with ε^{15}[A]. EOP of ε^{15}[A] on cells of A(ε^{34}) is about 1/3 of that on A. Cells A(ε^{34}) were infected with ε^{15}[A] at m.o.i. of 5-10. Surviving cells were plated out 20-30 minutes after infection and resulting colonies were tested for phage production by replica plating. As shown in Table 1, the proportion of lysogenic colonies among survivors was smaller than that calculated from the number of cells infected theoretically. This suggests that injected DNA of phage ε^{15}[A] may be destroyed some time after infection. Similar suggestion comes from the fact that 100% of cells A recover from infection with ε^{15}[I-1] (Table 6).

m. Rescue of ε^{15}vir[A] by superinfecting ε^{15}ts[A]. If injected phage DNA were destroyed, it would be expected that the longer the time elapses before adding a second rescuing phage, the lower the efficiency of rescuing a primary restricted phage would be.

Growing cells of I-1 were infected with ε^{15}vir[A] at low m.o.i. and at appropriate time intervals thereafter superinfected with ε^{15}ts[A] at high m.o.i. to see the efficiency of rescuing ε^{15}vir. As seen in Fig. 10, the results showed that the efficiency of rescuing ε^{15}vir decreases with prolongation of time intervals between primary and secondary infections, suggesting the destruction of injected DNA of primary phage ε^{15}vir[A].

n. Effects on EOP of heating cells in HCV. What factor is responsible for the destruction of DNA of the restricted phage? To look

into the nature of the responsible factor, heat effect was tested.

Growing cells of I-1 were heated in water bath for 30 seconds -- 5 minutes at temperatures ranging from 44°C to 55°C, transfered back to 37°C, kept for 1-2 minutes, and infected with ε^{15}[A] at low m.o.i.. As shown in Fig. 11, EOP increased remarkably by heating at 49-50°C for 2 minutes. This suggests that the responsible factor is thermolabile.

o. Effects on EOP of multiple infection of A with ε^{15}[I-1]. In infection of cells A with ε^{15}[I-1], MA effect was not so remarkable. At m.o.i. of about 50, plaque formers increased by 10 times. But even under this condition, the proportion of plaque formers to infected cells is only 10^{-3}.

p. Summary and Conclusions. Under the conditions employed, phage ε^{15} propagated on one strain shows lower EOP on the other, and this reciprocal shifting of EOP proved to be due to HCV by experiments described in c through o.

ε^{15}[A] and ε^{15}[I-1] are serologically indistinguishable. By infection phage genetic material is injected into the host cell regardless of EOP. Only the fate of injected DNA seems to be different and abnormal when injected into cells on which phage shows lower EOP. Among the data presented, most remarkable is MA. The results showed clearly that cells of I-1 which do not allow multiplication of ε^{15}[A] by single infection may allow phage multiplication when multiply infected. As for the mechanism of MA, following possibilities may be considered: (1) Genetic cooperation or complementation; (2) genetic recombination; (3) removal of inhibitors of phage multiplication.

ε^{15}ts is characteristic in that it is unable to multiply at 37°C. Nevertheless ε^{15}ts[A] is capable of contributing to MA in infection of I-1 with ε^{15}[A] while ε^{15}ts[I-1] is incapable (k). This favors the hypothesis (3) but not (1) or (2).

On the other hand, the fact that UV-inactivated ε^{15}[A] is incapable of contributing to MA (j) suggests that the intact structure of ε^{15}[A] DNA is necessary for MA. And the fact that ε^{15}[I-1] or ε^{15}ts[I-1] takes little or no MA effect for ε^{15}[A], suggests that the helper effect is controlled by only a minor difference in DNA structure.

In addition, the specific destruction of DNA of restricted phage is suggested (l), and the factor(s) responsible for this destruction can be overcome by multiple infection (Section 2-f) and is thermolabile (n).

Summing up the above findings, the factor(s) responsible for HCV seems to be thermolabile, highly specific, capable in interacting with intact phage DNA, and present in a small amount in a cell. If the factor(s) with such properties is assumed to be a DNase, all the

data could be explained quite easily. The experiments along these lines are under way, and some of preliminary experiments have shown the results favoring the hypothesis but the details will be described in future reports. Recent papers on phage λ (Arber and Dussoix, 1962; Dussoix and Arber, 1962) seem to be along similar line.

4. Isolation of mutants of the converting phage ε^{15}

For carrying out experiments on HCV and others, it became desirable to isolate mutants of the phage ε^{15}, since a few mutants with abnormal converting properties were on hand but they were found not to be adequate for these purposes.

a. Materials and Methods

(1) Bacterial and phage strains, anti-ε^{15} serum, antibacterial antiserum, anti-10 and anti-15 sera are described in Sections 2 and 3.

(2) Physiological saline, phosphate buffer, and M-9 salt solutions were sterilized before use.

(3) Procedures for slide and tube agglutination tests, and test for lysogenicity are also described in Sections 2 and 3.

(4) Phage adsorption, neutralization, single-step growth experiment were done by routine procedures (Adams, 1959).

(5) Bresch's medium (Bresch, 1952; Bresch und Trautner, 1956) was employed for selecting plaque type mutants.

(6) Mutagenic substances. Ethyl methane sulfonate (Bautz and Freese, 1960; Strauss and Okubo, 1960; Green and Krieg, 1961; Strauss, 1962), nitrous acid (Gierer and Mundry, 1958; Boeyé, 1959; Tessman, 1959; Vielmetter und Wieder, 1959; Sinsheimer, 1960; Vielmetter and Schuster, 1960; Bautz-Freese and Freese, 1961; Granoff, 1961; Baylor and Mahler, 1962), 5-bromouracil (Brenner et al., 1959; Litman and Pardee, 1956, 1959, 1960a, 1960b), and 5-bromodeoxyuridine (Freese, 1959a, 1959b; Lawley and Brookes, 1962) were employed as chemical mutagens.

b. Search for plaque type mutants. Attempts were made to isolate plaque type mutants of the phage ε^{15} by using Bresch's medium, adding as one of ingradients one of carbohydrates such as dulcitol, i-inositol, inulin, maltose, d-mannose, raffinose, rhamnose, trehalose, d-xylose, and l-arabinose. A few plaque type mutants were isolated, but the difference from each other and from wild type of plaque was not remarkable that they were not useful in practice.

c. Isolation of temperature sensitive mutants with ethyl methane

sulfonate. Phages ε^{15}[A] were exposed to ethyl methane sulfonate (0.4M; 0.6M; 1M) in broth and or in M-9 salt solution at 25°C for 4 hours. At a concentration of 0.4M for 4 hours, about 95% of phages were inactivated. Surviving phages were propagated on cells A at 25°C for 3 hours for single-step growth. Released phages were plated at 25°C and each of resulting isolated plaques was transfered in duplicate onto plates seeded with cells A, of which one was incubated at 37°C and the other at 25°C, and phage strains which gave rise to plaque at 25°C but not at 37°C were selected out. Only two such strains (ε^{15}ts) were isolated among 9080 plaques examined.

d. Properties of mutant phage ε^{15}ts. ε^{15}ts is able to multiply at 25°C but not at 37°C. At 37°C ε^{15}ts-infected cells are either killed without lysis and phage release, or lysogenized. The proportion of surviving cells among ε^{15}ts-infected cells is larger at 25°C than at 37°C when compared at the same m.o.i.. Even at 37°C cells surviving infection segregate ε^{15}ts-carrier and non-carrier cells in their progeny for about 3 hours after infection, by which time the establishment of lysogeny is completed, as observed in the case of infection of cells A with wild type of phage ε^{15}ts$^+$ (see Section 2).

When ε^{15}ts-infected cells, which are to be killed and kept at 37°C, are transfered to 25°C before the end of rise period, phage multiplication may be restored. This restoration phenomenon is now under further investigation and the details will be described in future reports.

When stored in broth and/or physiological saline, phage ε^{15}ts is as stable as ε^{15}ts$^+$. In broth inactivation was not observed to a demonstrable extent for 6 months at 0°C-5°C. It is resistant to chloroform as the wild type is. Plaques produced by ε^{15}ts are indistinguishable from those by ε^{15}ts$^+$. The adsorption rate constant of ε^{15}ts onto cells A is 2.5×10^{-9}ml min^{-1} at 37°C and 2.3×10^{-9}ml min^{-1} at 25°C. ε^{15}ts is neutralized to the same extent as ε^{15}ts$^+$ by antiphage serum against ε^{15}ts$^+$. Single-step growth experiments at 25°C showed that the latent period is about 80 minutes, the rise period 100-110 minutes, and the average burst size about 600.

The spontaneous back mutant was found $4/10^5$ in one strain and $3/10^4$ in the other.

The antigen converting property of ε^{15}ts is the same as that of ε^{15}ts$^+$. Strain A(ε^{15}ts) was obtained by lysogenizing strain A with phage ε^{15}ts. By both slide and tube agglutination tests, and by agglutinin absorption test, A(ε^{15}ts) was confirmed to possess antigens 3.15 as somatic antigens, the same structure as that of A(ε^{15}ts$^+$) No antigenic difference was found between cells A(ε^{15}ts) grown at 25°C and those grown at 37°C.

ε^{15}ts has also proved to be very useful for the researches on HCV as already described in Section 3.

e. Induction of mutations in phage ε^{15} with nitrous acid, 5-bromouracil, and 5-bromodeoxyuridine. By treating phage ε^{15} with nitrous acid (5×10^{-2}M), 5-bromouracil (50 μg/ml) and or 5-bromodeoxyuridine (250 μg/ml), mutants, especially of plaque type, have been searched for. Several strains which give rise to larger plaques than that by wild type of ε^{15} have been isolated and their properties are now under investigation in detail.

f. Summary. Search for mutants of ε^{15}, which are useful for carrying out experiments on HCV and others, has been attempted by using Bresh's color medium with various carbohydrates and or by chemical mutagens.

Temperature sensitive mutant strains ε^{15}ts were isolated after treating ε^{15} with ethyl methane sulfonate. Their properties are as described in 4-d, among which most characteristic is their inability to multiply at 37°C. And on account of this special character, ε^{15}ts has provided a very useful tool for the researches into the mechanism of HCV as described in Section 3.

Several other mutants have also been isolated with the use of other chemical mutagens.

5. Chemical structure of somatic antigens and phage receptors in bacterial strains.

Our analysis of monosaccharide composition in specific somatic antigens of group E_1 (3.10), E_2(3.15), E_3 ((3)(15)34), and E_4 (1.3.19) revealed that they are qualitatively indistinguishable from each other, showing glucose, galactose, mannose, rhamnose, xylose and glucosamine (Ise, 1954; Nakagawa, 1954; Sasaki, 1955, 1956a, 1956b). These also seemed to suggest that different specificities of somatic antigens might be determined by different configurations of polysaccharide chains. And further studies have been being carried out to clarify the structures of determinant groups, in collaboration with the Massachusetts Institute of Technology, Cambridge, U. S. A., where we sent Dr. T. Uchida, one of our collaborators. Receptors for phages ε^{15}, C_{341}, and or ε^{34} are associated with somatic antigens 3.10 or 3.15 but their exact properties remain to be determined.

Since Uchida, with collaboration of Dr. P.W. Robbins, has clarified the structure of determinant groups of antigens 10, 15 and 34, our main concern at present has been directed toward the structure of antigen 7. And for this purpose, strain 87Aa', a mutant of Salmonella senftenberg (Ise, 1954), was employed, since it possesses factor 3 alone, which is common to group E Salmonellas, as somatic antigen. Although researches are on the way and the results obtained are preliminary, the followings have been observed so far.

a. Materials and Methods

(1) Bacterial strains: Strains A, A(ε^{15}), A(ε^{34}), and A(ε^{15},ε^{34}) are described in Section 2. Strains 87A and 87Aa' of Salmonella senftenberg were reported by Ise (1954).

(2) Bacterial cells: Strain 87Aa' was grown in nutrient broth enriched with yeast extract under aeration, and bacterial cells were harvested by Sharples centrifuge.

(3) Antibacterial antiserum, anti-10 and anti-15 sera are described in Section 3. Anti-87Aa' O serum was also employed.

b. Extraction and purification of antigenic polysaccharide. A crude lipopolysaccharide (LPs) was prepared from cells of 87Aa' by hot phenol extraction (Westphal et al., 1952), dialysis of the aqueous phase, and repetitive acetone precipitation, followed by lyophilization. The crude LPs preparation was separated by ultracentrifuge into sediment and supernatant, both of which contained LPs and a small amount of protein. This protein was removed by passing through anion exchange resin column. The supernatant fraction showed a strong absorption at 260 mu, indicating the presence of nucleic acid, while the sediment fraction showed no absorption at 260 mu, being free from nucleic acid. The nucleic acid moiety in LPs-NA was confirmed to be of ribonucleic acid type by paper chromatography and by fractionation method of Schmidt-Thannhauser. Among several attempts to separate RNA from LPs in LPs-RNA preparation, only alkali hydrolysis was found to be effective. These findings are suggestive concerning the type of linkage between LPs- and RNA-moieties in the LPs-RNA fraction. Both fractions are reactive with anti-87Aa' O serum to a similar extent, containing glucose, galactose, mannose, rhamnose and a trace of xylose as monosaccharide components. An additional monosaccharide component, ribose, was found in LPs-RNA fractions.

c. Separation of constituent oligosaccharides from partial acid hydrolysate of nucleic acid-free LPs. LPs was hydrolysed partially with N-sulfuric acid at 100°C for 20 minutes. Hydrolysate was neutralized with $Ba(OH)_2$, centrifuged and concentrated for charcoal-celite chromatography. Fractionation was carried out by H_2O-ethanol gradient elution, and the sugar content in each of separated fractions was measured by phenol-sulfuric acid (Fig. 12). Paper chromatography revealed that fractions 5, 6, 7, and 8 contain separable oligosaccharides, although overlapping each other. Fractions 12 through 18 also contain oligosaccharides indistinguishable by paper chromatography. It is noteworthy that galactose, mannose, and rhamnose are main constituent monosaccharides in partial hydrolysate (peaks 3 and 4) and even in complete hydrolysate. These findings are suggestive in that main structure of polysaccharide would be composed of these three monosaccharides.

6. List of papers submitted to publication under the support from the Contract No DA-92-557-FEC-35775

Uetake, H. and Hagiwara, S.: Effects of the unrelated phage upon lysogenization. Virus (in Japanese)

Hagiwara, S., Uetake, H. and Toyama, S.: Lysogenization and host-controlled variation. Virus (in Japanese)

Uetake, H., Toyama, S. and Hagiwara, H.: Host-controlled variation in Salmonella phage ε^{15}. Virus (in Japanese)

Uetake, H., Toyama, S. and Hagiwara, S.: Host-controlled variation and multiplicity activation. Virus (in Japanese)

REFERENCES

Adams, M.H.: Bacterial phages (1959) Interscience Publ. New York.

Anderson, E.S. & Felix, A.: Variation in Vi-phage II of Salmonella typhi. Nature 170, 492-494 (1952)

Anderson, E.S. & Felix, A.: The Vi-type determining phages carried by Salmonella typhi. J.Gen.Microbiol. 9, 65-88 (1953)

Arber, W. & Dussoix, D.: Host specificity of DNA produced by Escherichia coli. I. Host controlled modification of bacteriophage λ. J.Mol.Biol. 5, 18-36 (1962)

Arber, W. & Lataste-Dorolle, C.: Erweiterung des Wirtbereiches des Bakteriophagen auf Escherichia coli B. Pathol.Mikrobiol. 24, 1012-1018 (1961)

Baricelli, N.A.: An analytical approach to the problems of phage recombination and reproduction. I. Multiplicity reactivation and the nature of radiation damages. Virology 11, 99-135 (1960)

Bautz, E. & Freese, E.: On the mutagenic effect of alkylating agent. Proc.Natl.Acad.Sci., 46, 1585-1594 (1960)

Bautz-Freese, E. & Freese, E.: Induction of reverse mutations and cross reactivation of nitrous acid-treated phage T4. Virology 13, 19-30 (1961)

Baylor, M.B. & Mahler, H.R.: Effects of nitrous acid on intracellular T2 bacteriophage. Virology 16, 444-451 (1962)

Bertani, G.: Lysogeny. Advances in Virus Research 5, 151-193 (1958)

Bertani, G.: Multiple lysogeny from single infection. Virology 18, 131-139 (1962)

Bertani, G. & Weigle, J.J.: Host-controlled variation in bacterial viruses. J.Bacteriol. 65, 113-121 (1953)

Boyce, A.: Induction of a mutation in poliovirus by nitrous acid. Virology 9, 691-700 (1959)

Brenner, S. & Smith, J.D.: Induction of mutations in the deoxyribonucleic acid of phage T2 synthesized in the presence of chloramphenicol. Virology 8, 124-125 (1959)

Bresch, C.: Unterscheidung verschiedener Bakteriophagentypen durch Farbindikatornährboden. Zbl.Bakt. I Orig. 159, 47 (1952)

Bresch, C. u. Trautner, T.: Die Bedeutung des Zweifarb-Nährbodens für genetische Untersuchungen am Bakteriophagen T1. Zsch.Indukt. Abstammungs- und Vererbungslehre. 87, 590-594 (1956)

Christensen, J.R.: The fate of genes from restricted T1 in lysogenic Shigella dysenteriae, Sh(P1). Virology 16, 133-139 (1962)

Christensen, J.R.: On the process of host-controlled modification of bacteriophage. Virology 13, 40-43 (1961)

Drexler, H. & Christensen, J.R.: Genetic crosses between restricted and unrestricted phage T1 in lysogenic and nonlysogenic hosts. Virology 13, 31-39 (1961)

Dussoix, D. & Arber, W.: Host specificity of DNA produced by Escherichia coli. II. Control over acceptance of DNA of bacteriophage λ. J.Mol.Biol. 5, 37-49 (1962)

Epstein, R.H.: A study of multiplicity-reactivation in the bacteriophage T4. Virology 6, 382-404 (1958)

Felix, A. & Anderson, E.S.: Bacteriophages carried by the Vi-phage types of Salmonella typhi. Nature 167, 603 (1951)

Freese, E.: The difference between spontaneous and base-analogue induced mutations of phage T4. Proc.Natl.Acad.Sci. U.S. 45, 622-633 (1959a)
Freese, E.: The specific mutagenic effect of base analogues on phage T4. J.Mol.Biol. 1, 87-105 (1959b)
Garen, A. & Zinder, N.D.: Radiological evidence for partial genetic homology between bacteriophage and host bacteria. Virology 1, 347-376 (1955)
Gierer, A. & Mundry, K.W.: Production of mutants of Tobacco mosaic virus by chemical alteration of its ribonucleic acid in vitro. Nature 182, 1457-1458 (1958)
Granoff, A.: Induction of Newcastle disease virus mutants with nitrous acid. Virology 13, 402-408 (1961)
Green, D.M. & Krieg, D.R.: The delayed origin of mutants induced by exposure of extracellular phage T4 to ethyl methane sulfonate. Proc.Natl.Acad.Sci. 47, 64-72 (1961)
Hagiwara, S.: Analysis of the process of lysogenization in phage ε^{34} system (I). Segregation of nonlysogenic progeny. Virus 9, 468-472 (1959a) (in Japanese)
Hagiwara, S.: Analysis of the process of lysogenization in phage ε^{34} system (II). Appearance of O antigen 34 in freshly phage-infected cells. Virus 9, 472-475 (1959b) (in Japanese)
Hagiwara, S.: Analysis of the process of lysogenization in phage ε^{34} system (III). Superinfection resistance in phage-infected cells and their progenies. Virus 9, 531-535 (1959c) (in Japanese)
Harm, W.: On the mechanism of multiplicity reactivation in bacteriophage. Virology 2, 559-564 (1956)
Ise, T.: Changes induced in the O antigens of Salmonella senftenberg and chemical analysis of specific O antigens. III. Monosaccharide composition of specific antigenic polysaccharides of the induced variant and summary of three reports. Sapporo Med.J. 5, 101-106 (1954) (in Japanese)
Kauffmann, F.: Enterobacteriaceae (1954), Munksgaard, Copenhagen
Lawley, P.D. & Brookes, P.: Ionization of DNA bases or base analogues as a possible explanation of mutagenesis, with special reference to 5-bromodeoxyuridine. J.Mol.Biol. 4, 216-219 (1962)
Lederberg, J. & Lederberg, E.M.: Replica plating and indirect selection of bacterial mutants. J.Bacteriol. 63, 399-408 (1952)
Litman, R.M. & Pardee, A.B.: The induction of mutants of bacteriophage T2 by 5-bromouracil. III. Nutritional and structural evidence regarding mutagenic action. Bioch.Biophys.Acta 42, 117-130 (1960a)
Litman, R.M. & Pardee, A.B.: The induction of mutants of bacteriophage T2 by 5-bromouracil. IV. Kinetics of bromouracil-induced mutagenesis. Bioch.Biophys.Acta 42. 131-140 (1960b)
Litman, R.M. & Pardee, A.B.: Mutations of bacteriophage T2 induced by bromouracil in the presence of chloramphenicol. Virology 8, 125-127 (1959)
Litman, R.M. & Pardee, A.B.: Production of bacteriophage mutants by a disturbance of deoxyribonucleic acid metabolism. Nature 178, 529-531 (1956)
Luria, S.E.: Host-induced modifications of viruses. Cold Spring Harbor Symp.Quant.Biol. 18, 237-244 (1953)

Luria, S.E.: Reactivation of irradiated bacteriophage by transfer of self-reproducing units. Proc.Natl.Acad.Sci. U.S., 33, 253-264 (1947)

Luria, S.E. & Delbecco, R.: Genetic recombination leading to production of active bacteriophage from ultraviolet inactivated bacteriophage particles. Genetics 34, 93-125 (1949)

Luria, S.E., Fraser, D.K., Adams, J.N. & Burrous, J.W.: Lysogenization, transduction, and genetic recombination in bacteria. Cold Spring Harbor Symp.Quant.Biol. 23, 71-82 (1958)

Luria, S.E. & Human, M.L.: A nonhereditary, host-induced variation of bacterial viruses. J. Bacteriol. 64, 557-569 (1952)

Maio, J.J. & Zahler, S.A.: Observations on the host-induced modification of T2 bacteriophage with special reference to the effects of α-keto acids. J.Bacteriol. 76, 256-263 (1958)

Nakagawa, A.: Lysogenic conversion of O antigen 34 in Group E Salmonella (I). Jap.J.Bacteriol. 12, 46-52 (1957a) (in Japanese)

Nakagawa, A.: Lysogenic conversion of O antigen 34 in Group E Salmonella (II). Jap.J.Bacteriol. 12, 107-110 (1957b) (in Japanese)

Nakagawa, A.: Lysogenic conversion of O antigen 34 in Group E Salmonella (III). Jap.J.Bacteriol. 14, 693-697 (1959)

Nakagawa, T.: Changes induced in the antigens of Salmonella (V). Chemical analysis of antigenic polysaccharide of Salmonella E_2 group. Sapporo Med.J. 5, 220-223 (1954) (in Japanese)

Ralston, D.J. & Krueger, A.P.: Phage multiplication on two hosts. Isolation and activity of variants of Staphylococcus phage P1. Proc.Soc.Exptl.Biol.Med. 80, 217-220 (1952)

Sasaki, T.: Chemical analysis of bacterial polysaccharides. I. Studies on the monosaccharide constituents of the specific polysaccharide of O antigens of Salmonella anatum (Group E_1). Preparations and purifications of the antigenic polysaccharides. Jap.J.Bacteriol. 10, 997-1002 (1955) (in Japanese)

Sasaki, T.: Chemical analysis of bacterial polysaccharides. II. Studies on the monosaccharide constituents of the specific polysaccharide of O antigens of Salmonella anatum (Group E_1). Monosaccharide constituents of the antigenic polysaccharides. Jap.J.Bacteriol. 11, 11-15 (1956a) (in Japanese)

Sasaki, T.: Chemical analysis of bacterial polysaccharides. III. Studies on the monosaccharide constituents of the specific polysaccharide of O antigens of Salmonella senftenberg (Group E_3). Jap.J.Bacteriol. 11, 107-110 (1956b) (in Japanese)

Sinsheimer, R.L.: The biochemistry of genetic factors. Ann.Rev. Biochem. 29 503-524 (1960)

Stent, G.S. & Fuerst, C.R.: Decay of incorporated radioactive phosphorus during development of a temperate bacteriophage. Virology 2, 737-752 (1956)

Strauss, B.S.: Response of Escherichia coli auxotrophy to heat after treatment with mutagenic alkyl methane sulfonates. J.Bacteriol. 83, 241-249 (1962)

Strauss, B.S. & Okubo, S.: Protein synthesis and the induction of mutations in Escherichia coli by alkylating agents. J. Bacteriol. 79, 464-473 (1960)

Tessman, I.: Mutagenesis in phages ØX174 and T4 and properties of the genetic material. Virology 9, 375-385 (1959)
Tessman, I. & Ozaki, T.: Multiplicity reactivations of bacteriophage T1. Virology 4, 315-327 (1957)
Uchida, T., Nakagawa, A. & Kai, M.: Variation of the surface structure of bacterial cell by lysogenization. Sapporo Med.J. 9, 268-271 (1956) (in Japanese)
Uetake, H.: Lysogenic conversion in bacterial antigens. Proc.Intern. Genetics Symp.Tokyo & Kyoto, 645-650 (1956)
Uetake, H. & Hagiwara, S.: Genetic cooperation between unrelated phages. Virology 13, 500-506 (1961)
Uetake, H. & Hagiwara, S.: Lysogenization and nuclear segregation. Virus 9, 574-577 (1959) (in Japanese)
Uetake, H. & Hagiwara, S.: The relationships between somatic antigens 15 and 34 in Salmonella. Virus 10, 150-154 (1960b) (in Japanese)
Uetake, H. & Hagiwara, S.: Somatic antigen 15 as a precursor of antigen 34 in Salmonella. Nature 186, 261-262 (1960a)
Uetake, H., Luria, S.E. & Burrous, J.W.: Conversion of somatic antigens in Salmonella by phage infection leading to lysis or lysogeny. Virology 5, 68-91 (1958)
Uetake, H. & Uchida, T.: Mutants of Salmonella phage ε^{15} with abnormal conversion properties. Virology 7, 495-505 (1959)
Vielmetter, V.W. & Schuster, H.: The base specificity of mutation induced by nitrous acid in phage T2. Biochem.Biophys.Research Communs. 2, 324-328 (1960)
Vielmetter, V.W. & Wieder, C.M.: Mutagene and inaktivierende Wirkung salpetriger Säure auf freie Partikel des Phagen T2. Z.Naturforsch. 14b, 312-317 (1959)
Westphal, O.: Ueber die Extraktion von Bakterium mit Phenol/Wasser. Z.Naturforsch. 7b, 148-155 (1952)

APPENDIX "A" TABLE

Table 1 Time required for establishing lysogeny at various multiplicities of infection in various combinations among bacterial strains

Bacterial strain	Phage	m.o.i.	Surviving cells* (%)	ε^{15}-producing colonies (%)	Prophage state was established (in hours)
A	ε^{15}[A]	1.05	47.1	43.3	ca 3
		5.0	35.0	99.9	ca 3
A(ε^{34})		6.1	26.1	44.8	>6
		7.5	25.0	51.2	>6
		8.9	36.7	86.7	>6
		20.0	65.5	98.7	ca 3
		24.2	74.6	98.9	ca 3
	ε^{15}[A(ε^{34})]	4.8	46.2	92.7	ca 3
		9.4	53.3	98.9	ca 3
		10.2	69.1	99.1	ca 3
Heated A(ε^{34})	ε^{15}[A]	3.9	23.3	33.6	>6
		5.0	21.9	42.9	>6
		15.0	17.1	97.3	ca 3-4
		15.4	20.2	98.1	ca 3-4

*Surviving cells were plated out 20 minutes after infection and the resulting colonies were tested for ε^{15} lysogenicity by replica plating.

Table 2 Efficiency of plating (EOP) of ε^{15} propagated on various strains and of g_{341}[A]

Phage strain	EOP on			
	A	A(ε^{34})	A(g_{341})	A(ε^{y})
ε^{15}[A]	1	3×10^{-1}	1	
ε^{15}[A(ε^{34})]	1	5×10^{-1}		
ε^{15}[A(g_{341})]	1		1	
g_{341}[A]	1	0		1

APPENDIX "A" TABLE

Table 3 Efficiency of plating and adsorption rate constant

ε^{15} propagated on	EOP		Adsorption rate constant ($\times 10^{-9}$ ml min^{-1})	
	A	I-1	A	I-1
A	1	10^{-2}	4.9	5.1
I-1	10^{-4}	1	5.1	4.3

A = S. anatum 293
I-1 = S. butantan

Table 4 One-cycle growth of ε^{15}[I-1] on cells A

	Indicator strain	
	A	I-1
Initial phages of ε^{15}[I-1]	3.7×10^{3}/ml	3.65×10^{7}/ml
Initial cells of A 1.04×10^{8}/ml		
Free phages after neutralization of free phages	0	2.0×10^{2}/ml
Phages adsorbed in 10 min		3.64×10^{7}/ml
m.o.i. = 0.35		
Plaque counts after burst	1.16×10^{5}/ml	1.0×10^{3}/ml
Burst size	31.4	

APPENDIX "A" TABLE

Table 5 Formation of antigen 15 on freshly phage-infected cells

Bacterial strain	Phage ε^{15}	Time after infection (min)	Dilution of anti-15 serum						Saline control
			1:80	1:160	1:320	1:640	1:1280	1:2560	
I-1	ε^{15}[I-1]	25	+	+	+	±	-	-	-
		50	+	+	+	±	-	-	-
A		25	+	+	+	-	-	-	-
		50	+	+	+	-	-	-	-
I-1	ε^{15}[A]	25	+	+	+	±	-	-	-
		50	+	+	+	+	-	-	-
A		25	+	+	+	-	-	-	-
		50	+	+	+	±	-	-	-
I-1			-	-	-	-	-	-	-
A			-	-	-	-	-	-	-
A(ε^{15})			+	+	+	+	+	-	-

+ = positive agglutination
- = no agglutination

Table 6 Proportion of cells killed or lysogenized by infection with host-controlled variants

Phage ε^{15} propagated on	Cells killed by phage (%)		Cells lysogenized among survivals (%)	
	A	I-1	A	I-1
A	66.4	92.6	99.8	30.0
I-1	0	85.0	0	60.5

APPENDIX "A" TABLE

Table 7 Mixed infection of cells I-1 with ε^{15}[A] and ε^{15}vir[A]

Cell input	1.01×10^8/ml	
ε^{15}[A] input	7.1×10^7/ml	
ε^{15}vir[A] input	7.2×10^7/ml	
Infective centers	2.78×10^6/ml	
Mixed yielders	1.8×10^5/ml	5.1×10^5/ml
Mixed(?) yielders	3.3×10^5/ml	
Calculated number of mixed yielders*	1.87×10^4/ml	

*Theoretical calculation was made by assuming that each phage suspension contains h mutant at a proportion of 10^{-2} and 2×10^{-2}, respectively.

APPENDIX "B" ILLUSTRATION

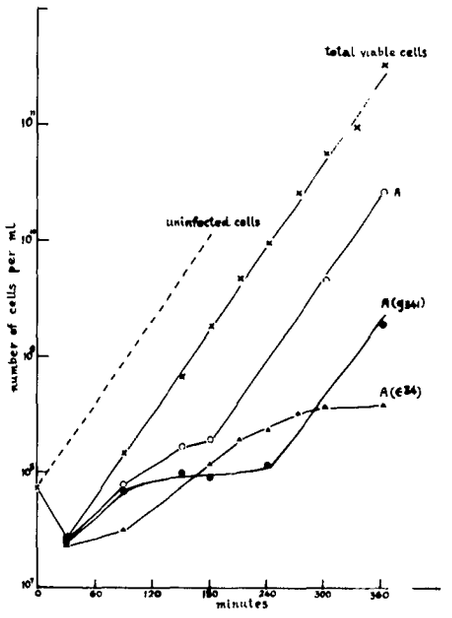

Fig. 1 Process of lysogenization in strains A, $A(\varepsilon_{341})$, and $A(\varepsilon^{34})$ infected with $\varepsilon^{15}[A]$

Growing cells of each strain were infected with $\varepsilon^{15}[A]$ and proportions of phage-carrier cells were followed up at appropriate time intervals.

Since experiments were not carried out at the same time, total viable cells in the experiment with cells A were taken, for comparison, as standard for viable cells, and phage-carrier cells were plotted according to the ratios of carrier to total cells.

APPENDIX "F" ILLUSTRATION

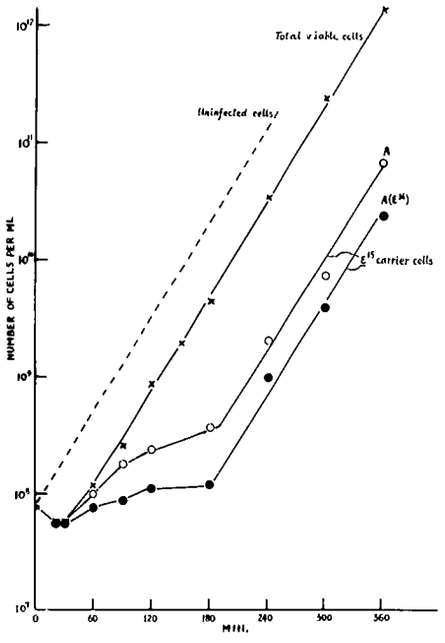

Fig. 2 Process of lysogenization in strains A and A(ε^{34}) infected with phage ε^{15}[A] at m.o.i. of 5 and 24.2, respectively

Growing cells of A(ε^{34}) were infected with ε^{15}[A] at m.o.i. of 24.2, while cells A as control at m.o.i. of 5.

APPENDIX "F" ILLUSTRATION

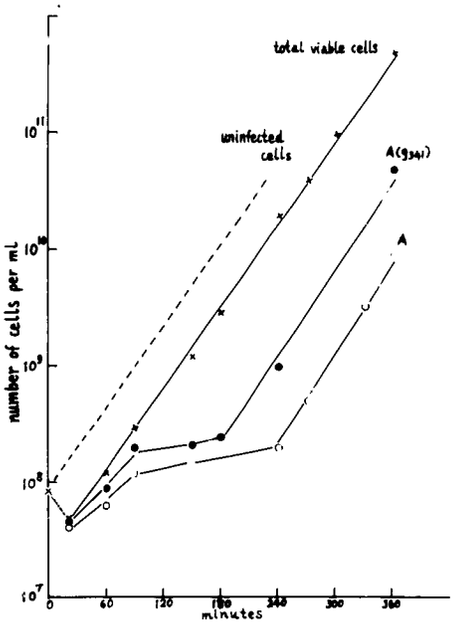

Fig. 3 Process of lysogenization in strains A and A(g_{341}) infected with ε^{15}[A(g_{341})]

Growing cells of A and A(g_{341}) were infected with ε^{15}[A(g_{341})] and proportions of phage-carrier cells were followed up at appropriate time intervals.

APPENDIX "B" ILLUSTRATION

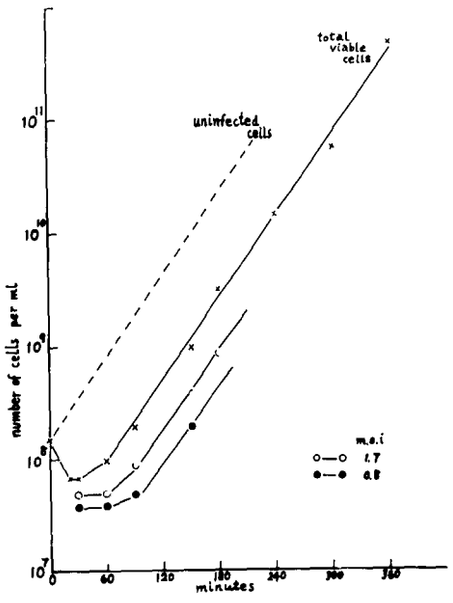

Fig. 4 Process of lysogenization in strain A infected with g_{341}[A]

Growing cells of A were infected with g_{341}[A] at various m.o.i., and proportions of phage-carrier cells were followed up at appropriate time intervals.

APPENDIX "B" ILLUSTRATION

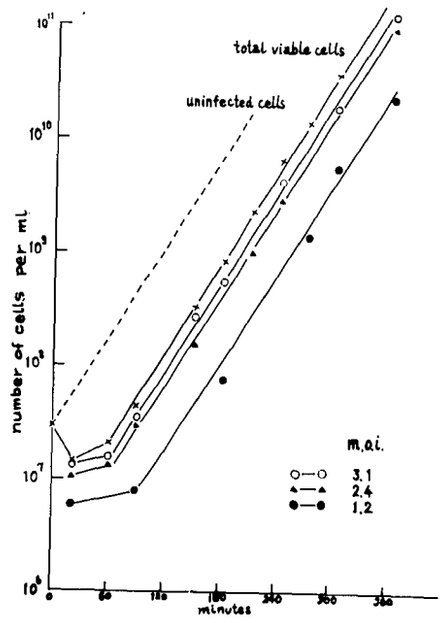

Fig. 5 Process of lysogenization in strain A(ε^y) infected with g_{341}[A]

Growing cells of A(ε^y) were infected with g_{341}[A] at various m.o.i., and proportions of phage-carrier cells were followed up at appropriate time intervals.

APPENDIX "B" ILLUSTRATION

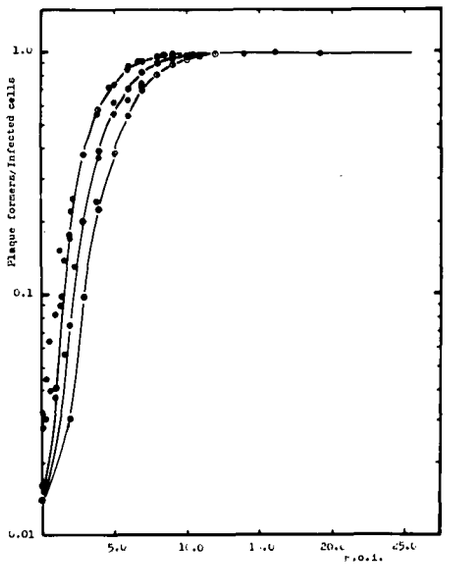

Fig. 6 Effect of multiple infection upon efficiency of plating (plaque formers/infected cells) in cells grown in nutrient broth

The proportion of plaque formers to infected cells was determined (•) at various m.o.i.. At each m.o.i., theoretical values were also calculated under the assumption that each cell may become plaque former, which receives at least 4(⊙), 5(⊙), and/or 6(⊙) phage particles, according to the formula shown in Fig. 7, where

Pr = proportion of plaque formers to infected cells
m = m.o.i.
r = number of phage particles adsorbed to a single cell
n = EOP in single infection

APPENDIX "B" ILLUSTRATION

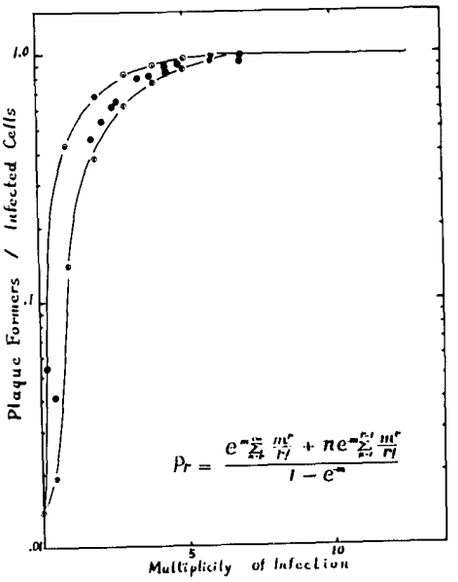

Fig. 7 Effect of multiple infection upon efficiency of plating (plaque formers/infected cells) in cells grown in synthetic medium

Notes are the same as in Fig. 6, except that calculations were made under the assumption that each cell may become plaque former, which receives at least 2(o), or 3(o) phage particles.

APPENDIX "B" ILLUSTRATION

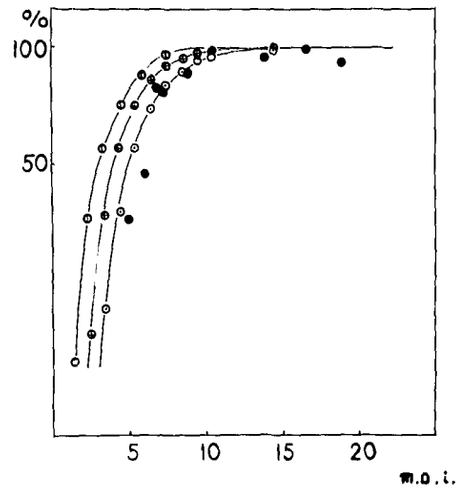

Fig. 8 Proportion of cells killed by phage infection at various m.o.i.

The proportions of killed to infected cells were determined (●) at various m.o.i.. At each m.o.i., theoretical values were also calculated, assuming that each cell may be killed by infection, which receives at least 4(○), 5(●), and/or 6(◎) phage particles.

APPENDIX "B" ILLUSTRATION

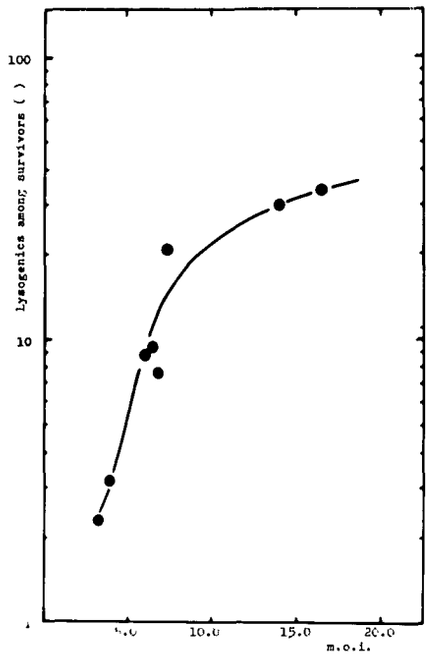

Fig. 9 Proportion of lysogenic cells among survivors at various m.o.i.

Phage-infected cells of I-1 were plated for surviving cells 20 minutes after infection, and resulting colonies were tested for lysogenicity by replica plating.

APPENDIX "B" ILLUSTRATION

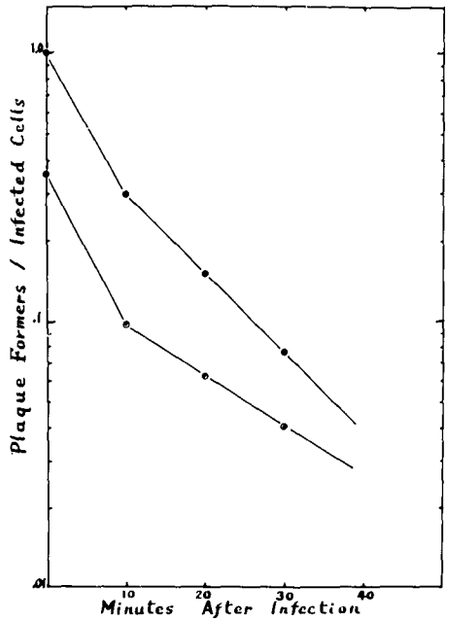

Fig. 10 Rescue of ε^{15}vir[A] by superinfecting with ε^{15}ts[A]

APPENDIX "B" ILLUSTRATION

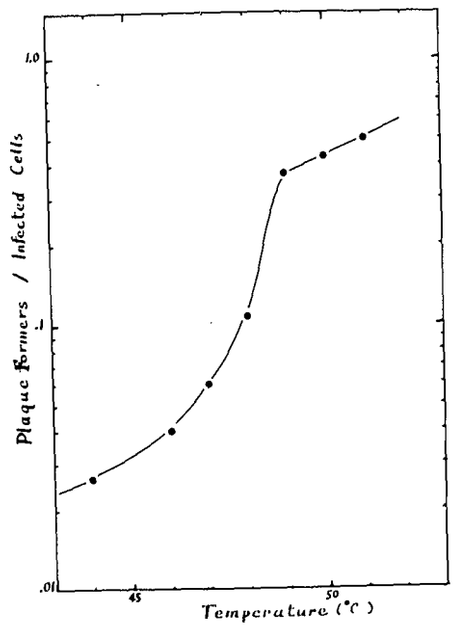

Fig. 11 Effect of heating cells on efficiency of plating (plaque formers/infected cells)

Growing cells of I-1 were heated for 2 minutes at various temperatures, transferred back to 37°C, infected with phage c^{15}[A] at low (< 0.1) m.o.i., and plated out for infective centers with cells of I-1 before lysis.

APPENDIX "B" ILLUSTRATION

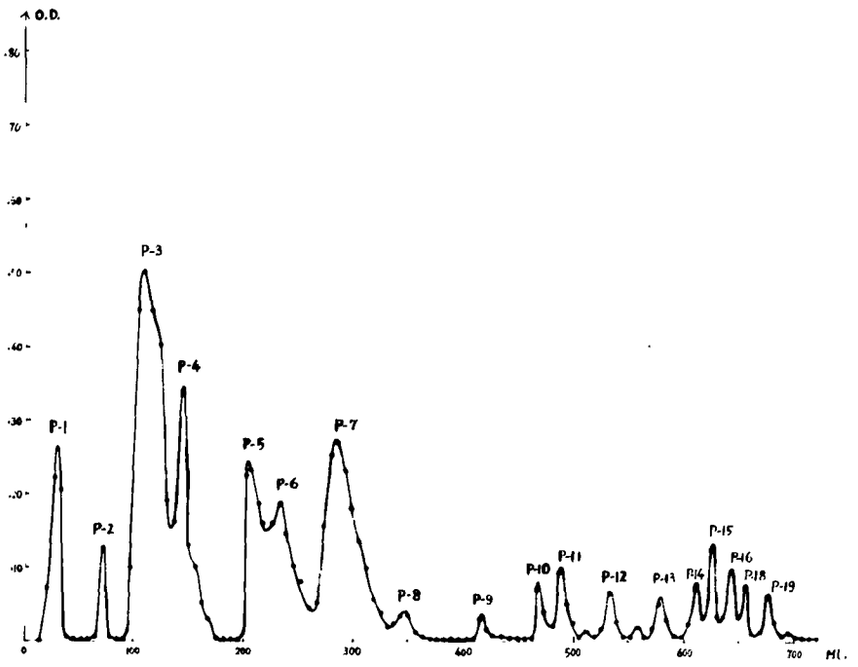

Fig. 12 Charcoal-celite column chromatography of acid hydrolysate of 87Aa' lipopolysaccharide

Partial acid hydrolysis: N H_2SO_4, 20 min.
Column: Acid-treated norit A(50 gm)/celite 545(100 gm)
Color development: Phenol-H_2SO_4 reaction